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16. ABSTRACT

Freshwater (Selenastrum capricornutum) and estuarine (Skeketonema costatum) algae were exposed to liquid wastes from 10 industrial sites in laboratory bioassays. All wastes affected algal growth wither by stimulation or by stimulation at low concentrations and inhibition at high concentrations. Generally S. capricornutum and Sk. costatum responded similarly to each waste: SC20's were between 0.01% waste; EC50's, between 5.1% and 85.5% waste. Toxicity to S. capricornutum was lost by the ninth day of exposure in all wastes except one, and it is recommended that algal tests be carried out for four days. Both algal species were more sensitive to the wastes than were Daphnia magna (freshwater) and Mysidopsis bahia (estuarine). Only three wastes were toxic to D. magna and two were toxic to D. magna and two were toxic to M. bahia SC20 and EC50 values are used to calculate the 7-day, 10-year flow rate required for dilution of effluents to non-toxic concentrations.

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ALGAE AND CRUSTACEANS AS INDICATORS OF BIOACTIVITY OF INDUSTRIAL WASTES*

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Abstract—Freshwater (*Selenastrum capricornutum*) and marine (*Skeletonema costatum*) algae were exposed to liquid wastes from 10 industrial sites in laboratory bioassays. All wastes affected algal growth, either by stimulation only or by stimulation at low concentrations and inhibition at high concentrations. Generally, *S. capricornutum* and *Sk. costatum* responded similarly to each waste: SC20's (concentration that stimulated growth by 20%) were between 0.01 and 20.0% waste; EC50's (concentration that inhibited growth by 50%), between 5.1 and 85.5% waste. Since toxicity to *S. capricornutum* was usually lost by the sixth or seventh day of exposure in all wastes except one, it is recommended that algal tests be carried out for 4 days. Both algal species were more sensitive to the wastes than were *Daphnia magna* (freshwater) and *Mysidopsis bahia* (marine). Only three wastes were toxic to *D. magna* and two were toxic to *M. bahia*. SC20 and EC50 values are used to calculate the 7-day, 10-year flow rate of the receiving stream required for dilution of effluents to non-toxic concentrations.

INTRODUCTION

Liquid industrial wastes are major sources of pollution in freshwater and estuarine systems. Thousands of industries throughout the world discharge effluents that contain mixtures of toxicants such as organic compounds and heavy metals, plant nutrients such as phosphate and nitrate, and a variety of other inorganic substances such as sodium chloride, ammonia, sulfates and sulfites. Properties of wastes such as pH and temperature may affect organisms in receiving waters. Joubert (1980) demonstrated inhibition of algal growth by water from a leachate site. Eloranta & Kettunen (1979) showed reduced algal biomass caused by low pH 15 km downstream from a sulfite cellulose factory, but increased algal biomass 15–50 km downstream from the outfall. Cain *et al.* (1979) showed that algal assay is a sensitive method for detection of pollution.

In general, aquatic animals are more sensitive than algae to single pollutants such as pesticides and heavy metals, but there is evidence that algae are more sensitive than animals to complex wastes, such as industrial and municipal effluents (Walsh *et al.*, 1980). In the study reported here, we determined effects of 10 industrial waste samples on *Selenastrum capricornutum*, a freshwater alga, and *Skeletonema costatum*, a marine alga. Effects on growth of these algae were compared to toxicity to crustaceans that are considered to be pollutant-sensitive species: *Daphnia magna* (freshwater) and *Mysidopsis bahia* (marine).

MATERIALS AND METHODS

Sampling of effluents was conducted at the outfalls of 10 industrial plants in Virginia and Maryland (Table 1) in the vicinity of Chesapeake Bay according to methods de-

Table 1. Industries from which samples were taken for algal and animal analyses

Effluent number	
100	Integrated paper mill; bleached Kraft and neutral sulfite semichemical process
101	Synthetic organic fibers
102	Textile dyeing and finishing of corduroy, velveteen, cotton and polyesters
103	Oil refinery producing gasoline and heating oils by thermal and catalytic cracking. Coke produced as byproduct
104	Textile mill: weaving, dying, printing and mechanical/chemical finishing
105	Integrated paper mill: semichemical process used to produced pulp from hardwood chips. Sanitary waste included in effluent
106 and 110	Integrated paper mill: Kraft process used to produce unbleached woodpulp
107	Resin and fibers: resin production, fiber spinning and drawing, fiber finishing. Production of nylon, acetate, acrylic and other fibers and resins
108	Leather tanning: tanning, curing and finishing of hides
109	Chemical manufacturing: major chemicals produced were sodium hydrosulfite, sodium metabisulfite, zinc sulfate and amines

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scribed in Environmental Protection Agency (EPA) (1977). A composite sample was taken at each site by an Isco automatic sampler and collected in a 190-l. volume acrylic plastic container. Composite sampling was begun by mid-morning and continued for 23–25 h. The sample was mixed, divided into approx. 20-l. portions in linear polyethylene containers, packed in ice and shipped by air freight to arrive at the testing laboratory less than 24 h after the final subsample was taken.

As a check on accuracy of the data, effluent from one industrial site was divided into two subsamples and numbered 106 and 110. Personnel at the testing laboratory were unaware that they were from the same site and the results of tests on the subsamples are reported.

Bioassays were conducted on freshwater organisms at the Battelle-Columbus Laboratories, Columbus, OH, and on marine organisms at Battelle's W.F. Clapp Laboratories, Duxbury, MA. Detailed methods are given in Duke *et al.* (1977), Walsh & Alexander (1980), and Walsh *et al.* (1980). Freshwater species were the green alga, *Selenastrum capricornutum* Printz, and the daphnid, *Daphnia magna* Straus. Marine species were the diatom, *Skeletonema costatum* (Greville) Clevel., and the mysid, *Mysidopsis bahia* Molenock. Algal tests contained three replications and animal tests contained two replications of each control and exposure concentration.

Exposure media for algae were prepared by adding salts, nutrients and vitamins to untreated waste and diluting to test concentrations with filter-sterilized freshwater growth medium prepared with well water (EPA, 1971) or autoclaved artificial sea-water of 30 ppt salinity prepared with deionized water (Walsh & Alexander, 1980).

The freshwater algal test was developed to correlate algal growth with the nutrient status of natural waters (EPA, 1971), but has also been used to detect toxicity (Joubert, 1980; Walsh *et al.*, 1980). Although a 14-day growth period is recommended (EPA, 1977), preliminary tests in our laboratory showed that toxicity is often lost in long-term algal bioassays. Therefore, numbers of *S. capricornutum* in each concentration of waste were estimated by fluorescence each day for 2 weeks. Fluorescence units were converted to cell numbers from regression charts. Standing crop of *S. capricornutum* after 14 days of exposure was estimated from dry weight of cells (EPA, 1977).

The concentration of waste that would stimulate freshwater algal growth by 20% (SC20) or inhibit growth by 50% (EC50) was derived by straight-line graphical interpolation (APHA, 1975). Growth rate was calculated from the expression

$$\mu = \frac{\ln(N_2/N_1)}{t_2 - t_1}$$

where μ = growth rate, N_1 = number of algae at the start of the test, N_2 = number of algae after 4 days, and $t_2 - t_1 = 4$ days. Duncan's Multiple Range Test was used to identify significant differences among growth rates and among final biomasses of treatment groups.

The marine algal growth test with *Sk. costatum* (EPA, 1977; Walsh & Alexander, 1980) recommends a 4-day exposure period, and toxicity and stimulation data are reported for that period only. Biomass on the fourth day was estimated by absorbance at 525 nm. The SC20 and EC50 were calculated by the method of Bahner & Oglesby (1980).

Responses of the algae were compared to responses of pollutant-sensitive freshwater and marine crustaceans exposured to the same wastes in static tests. The freshwater *D. magna* and marine *M. bahia* were exposed to each effluent according to methods given in EPA (1977). *Daphnia* was exposed to 100% waste or to lower concentrations prepared by dilution with filtered well water. *Mysidopsis* was exposed to 100% waste to which artificial sea-salts were added to a concentration of 30 ppt or to lower concentrations made by dilution with artificial sea-water prepared with deionized water. Response of both species in 4 days is expressed as the LC50, i.e. the calculated concentration that would kill 50% of the exposed population. The LC50 and 95% confidence limits were calculated by probit analysis (Finney, 1971; Stephan, 1977).

SC20 and LC50 values were used to calculate the dilution required to render each waste non-toxic or non-stimulatory to algae in receiving waters. The instream waste concentration (IWC) is an estimate of the amount of dilution that an effluent may be expected to undergo during continuous release into a receiving stream (EPA, 1977):

$$\text{IWC}(\%) = \frac{\text{Qw}}{\text{Qr} + \text{Qw}} \times 100\%$$

where Qw = volume of discharge, Qr = the 7-day, 10-year low-flow volume of the receiving water. According to current EPA regulations (EPA, 1979), if the IWC is equal to or greater than 0.01 times the LC50, then the discharge is considered hazardous. We have modified the above expression to include both the SC20 and LC50 because introduction of stimulatory or toxic substances to receiving waters is undesireable. Therefore, the minimum 7-day, 10-year low-flow volume (Qr) required for safety can be calculated from the expression

$$0.01 \times \text{LC50 or SC20} = \frac{\text{Qw}}{\text{Qr} + \text{Qw}} \times 100.$$

RESULTS AND DISCUSSION

Algae

A summary of the effluents tested is given in Table 1. They include wastes from 3 paper mills, 2 textile mills, 2 fiber plants, an oil refinery, a chemical manufacturing plant and a tannery. None was strongly inhibitory to algal growth, but all stimulated growth at low concentrations (Table 2). No waste type appeared to be either more or less bioactive than any other.

Samples 106 and 110, which were prepared by dividing a single effluent sample from a paper mill, yielded statistically similar results (Tables 2 and 4) and confirmed that the methods gave consistent results.

All of the wastes stimulated growth of both algal species. None was toxic without stimulation, as reported by Walsh & Alexander (1980) and Walsh *et al.* (1980). Wastes either stimulated growth at all concentrations (Fig. 1a), stimulated growth at all concentrations and were severely inhibitory at higher concentrations (Fig. 1b), or, in a response not reported before, were stimulatory at low concentrations, with less severe growth inhibition at higher concentrations. This last response was given by *Sk. costatum* with samples 100, 102 and 103 (Fig. 1c), but inhibition was not great enough to permit calculation of an EC50. In the example of *Sk. costatum* and Sample 100 given in Fig. 1(c), growth was inhibited by concentrations between 25 and 50% waste, and population density in 75% waste was approx. one-half that of the maximum attained in 25% waste.

There are, therefore, four types of response of *Sk. costatum* to complex industrial wastes: inhibition, with results expressed as the EC50; stimulation, with

Table 2. Effects of industrial wastes on growth of *Skeletonema costatum* and *Selenastrum capricornutum* after 4 days of exposure

	Sk. costatum		*S. capricornutum*	
Sample No.	SC20	EC50	SC20	EC50
100	0.07 (0.044–0.083)	t	0.04	62.1
101	1.0 (0.86–1.11)	21.2 (19.8–23.0)	1.96	45.6
102	0.53 (0.39–0.69)	t	0.01	NT
104	0.02 (0.006–0.049)	t	0.05	NT
104	0.64 (0.54–1.42)	73.0 (69.6–74.8)	0.01	NT
105	0.88 (0.55–1.20)	NT	0.91	13.6
106	0.73 (0.34–0.93)	63.5 (41.9–69.2)	0.02	28.6
107	0.04 (0.024–0.050)	NT	8.0	NT
108	1.03 (0.053–2.99)	29.9 (20.5–35.4)	1.0	5.1
109	20.0 (16.7–22.9)	44.3 (40.5–47.1)	0.4	81.5
110	0.49 (0.31–1.03)	42.4 (29.9–51.1)	0.09	43.4

SC20 = percentage waste that would stimulate growth by 20%; EC50 = percentage waste that would inhibit growth by 50%; NT = not toxic; NS = not stimulatory; t = toxicant present but not quantifiable; 95% confidence intervals are in parentheses; Samples 106 and 110 were from the same site.

results expressed as the SC20; stimulation and inhibition, with results expressed as the SC20 and EC50; and stimulation and inhibition, with stimulation expressed as the SC20 but with less severe inhibition expressed as it relates to the inflection point of the response curve.

Responses of *S. capricornutum* to exposure for four days were either enhanced growth alone or enhanced growth at low concentrations and growth inhibition at higher concentrations (Table 2, Fig. 2a). However, after 14 days of exposure, cell numbers and biomass in all treated cultures except one (Sample 108) were equal to or greater than those of the control in all concentrations. Measurement of standing crop was inappropriate for detection of toxicity (Table 3). Standing crop in each exposure concentration was greater than in the control after 14 days. However, 4-day growth rates demonstrated significant stimu-

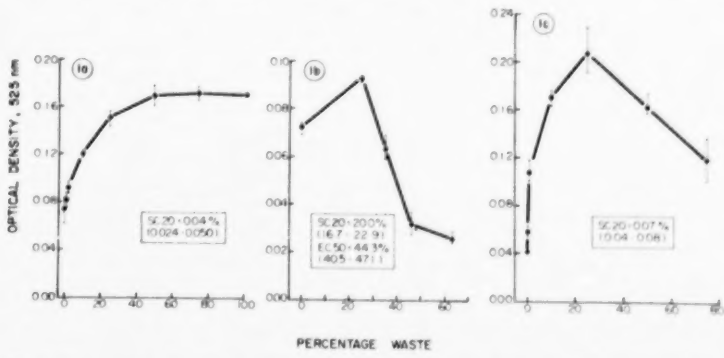

Fig. 1. Effects of industrial wastes on growth of *Sk. costatum*: (a) resin and fibers plant; (b) chemical manufacturing plant; (c) integrated paper mill. Vertical lines indicate range of optical densities; SC20 = calculated concentration (%) that would stimulate growth by 20%; EC50 = calculated concentration (%) that would inhibit growth by 50%; 95% confidence limits in parentheses.

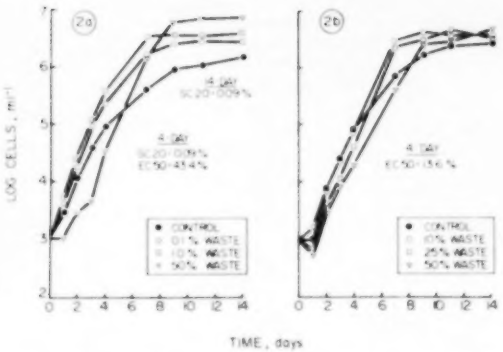

Fig. 2. Effects of wastes from integrated paper mills on growth of *S. capricornutum*: (a) Sample No. 106; (b) Sample No. 105. SC20 = calculated concentration (%) that would stimulate growth by 20%; EC50 = calculated concentration (%) that would inhibit growth by 50%.

lation at lower concentrations and significant inhibition at higher concentrations. Toxicity was lost, usually by the sixth or seventh day (Fig. 2b). From these tests, it is concluded that algal growth bioassays with complex industrial wastes need not be conducted for longer than 4 days.

There are several explanations why toxicity was lost. It is possible that as biomass of the algal populations increased and the ratio of biomass to toxicant concentration increased, effects were diminished. In our tests, even though the nutrient concentration of growth medium did not support maximal growth, cell densities in control cultures at 4 days were greater than those in natural systems. Waste concentrations used in these tests inhibited the rate of algal population growth, but did not kill the populations. Thus, it is possible that as the ratio of biomass to toxicant concentration increased, a critical point was reached beyond which there was no toxic effect and final population density was determined by nutrient content of the growth medium.

Other factors that could affect growth of algae in bioassays with industrial wastes are: adsorption of toxicants to walls of glass exposure vessels, degradation of toxicants by light required for algal growth and development of resistance to toxicants by the population early in the exposure period.

Because of these and possibly other factors that affect responses of algae to toxicants, algal tests carried out for only 4 days seem appropriate. Payne & Hall's (1979) test for algistatic response is inappro-

Table 4. Responses of *Daphnia magna* and *Mysidopsis bahia* to industrial wastes after 4 days of exposure in static tests

Sample No.	LC50, % Waste *M. bahia*	*D. magna*
100	NT	NT
101	44.9 (39.2–50.7)	NT
102	NT	77.5 (65.1–89.9)
103	NT	NT
104	45.4 (33.0–57.8)	NT
105	NT	NT
106	NT	NT
107	NT	NT
108	*	52.2 (44.6–59.8)
109	9.5 (8.0–11.4)	NT
110	NT	NT

LC50 = concentration that would kill 50% of the population in 4 days; NT = non-toxic; 95% confidence limits are given in parentheses; * = test invalid because of high mortality of control animals.

Table 3. Growth rates of *S. capricornutum* during 4 days of exposure and log of the standing crop after 14 days of exposure to effluents from a synthetic fibers plant (Sample 101) and an integrated paper mill (Sample 106)

Sample No.	Waste concentration (%)	Growth rate	Log of standing crop
101	0	0.508	6.21
	1	0.586(S*)	6.29(C)
	10	0.667(S†)	6.54(S*)
	25	0.648(S†)	6.51(S*)
	50	0.389(I*)	6.59(S*)
	100	0.259(I†)	6.46(S*)
106	0	0.360	6.10
	0.1	0.556(S†)	6.49(S†)
	1	0.603(S†)	6.65(S†)
	10	0.566(S*)	6.65(S†)
	25	0.403(C)	6.79(S†)
	50	−0.75(I†)	6.85(S†)

S = stimulation; I = inhibition; C = same as control; * = significant at 95% level; † = significant at 99% level.

Table 5. Minimum 7-day, 10-year low flow volumes (Qr) required to render effluents non-toxic based on the SC20 for algae and the LC50 for animals. NT = non-toxic

Effluent No.	Discharge rate $m^3\ day^{-1} \times 10^3$	Qr, $m^3\ day^{-1}$ *Sk. costatum*	*S. capricornutum*	*M. bahia*	*D. magna*
100	85.9	12.3×10^9	21.5×10^9	NT	NT
101	1.9	1.8×10^8	9.5×10^6	2.3×10^5	NT
102	4.5	8.4×10^7	4.5×10^9	NT	1.3×10^5
103	3.9	2.9×10^9	7.9×10^8	NT	NT
104	3.7	5.7×10^7	3.7×10^9	4.5×10^5	NT
105	19.0	2.1×10^8	2.1×10^8	NT	NT
106	40.7	5.5×10^8	2.0×10^9	NT	NT
107	22.5	5.6×10^9	2.6×10^7	NT	NT
108	0.8	7.9×10^6	7.9×10^6	NT	7.4×10^4
109	2.7	1.1×10^6	6.6×10^7	NT	2.5×10^5
110	40.7	8.3×10^8	4.5×10^9	NT	NT

priate for analysis of complex effluents because algae in receiving water are exposured continuously to them.

Animals

Daphnia magna and *Mysidopsis bahia* were less sensitive to the wastes than were the algae. Table 4 gives LC50 values for these crustaceans and shows that, whereas 9 wastes were toxic to *Sk. costatum* and 7 were toxic to *S. capricornutum*, only 3 were toxic to *D. magna* and 2 were toxic to *M. bahia*. The LC50 was higher than the EC50 for algae in every case except Samples 104 and 109 and the SC20 was always lower than the LC50. These data show that algal tests are better indicators of potential pollution from complex wastes than are animals because algae demonstrated possible roles of the effluents in eutrophication of receiving waters and responded more strongly to a greater number of effluents.

Flow requirements

Algal data were valuable in calculating the 7-day, 10-year low-flow rates required for safe disposal of the effluents. Table 5 shows that Qr values could be derived from the data for all samples and both algal species. Such values could be calculated for only 5 effluents with animal data, and each Qr value was lower than that calculated with algal data except for Sample 109. Algal data indicated need for greater dilution and thus afforded a greater margin of safety than did animal data.

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